Bovine DNA Proton and Alpha Particle Induced Damage Using XPS

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Additional and Supplementary Material

Papers involving XPS studies for Radiation-Induced DNA damage. None involving protons or α's at this point. So there is no connectivity in their results to SOBP effects and related factors such as depth variant and relatively large LET values

A) **XPS Study on DNA Damage by Low-Energ Electron Irradiation,** Hyung Ah Noh and Hyuck Cho, C Physics Department, JOURNAL OF RADIATION PROTECTION, VOL.36 NO.4 DECEMBER 2011 194

B) In situ monitoring of the influence of water on DNA radiation damage by near-ambient pressure X-ray photoelectron spectroscopy, Marc Benjamin Hahn, Paul M. Dietrich and Jörg Radnik , COMMUNICATIONS CHEMISTRY, https://doi.org/10.1038/s42004-021-00487-1

C) **Energy Thresholds of DNA Damage Induced by UV Radiation: An XPS Study** P. J. Gomes, A. M. Ferraria, A. M. Botelho do Rego, S. V. Hoffmann, P. A. Ribeiro, and M. Raposo, J. Phys. Chem. **B 2015**, 119, 5404−5411

Motivation for These Measurements

1) Does the change in the proton/α LET (dE/dx) have a significant effect on the type of molecular damage in DNA in terms of bond breakage of the constituent molecules re: nucleic acids, sugar and phosphate back bones?

2) Are there other mechanisms for bond breakage besides direct impact by the energetic ion on the constituent DNA molecules that cause bond breakage? For example does an aqueous environment add to bond breakage as opposed to irradiation of DNA crystals directly?

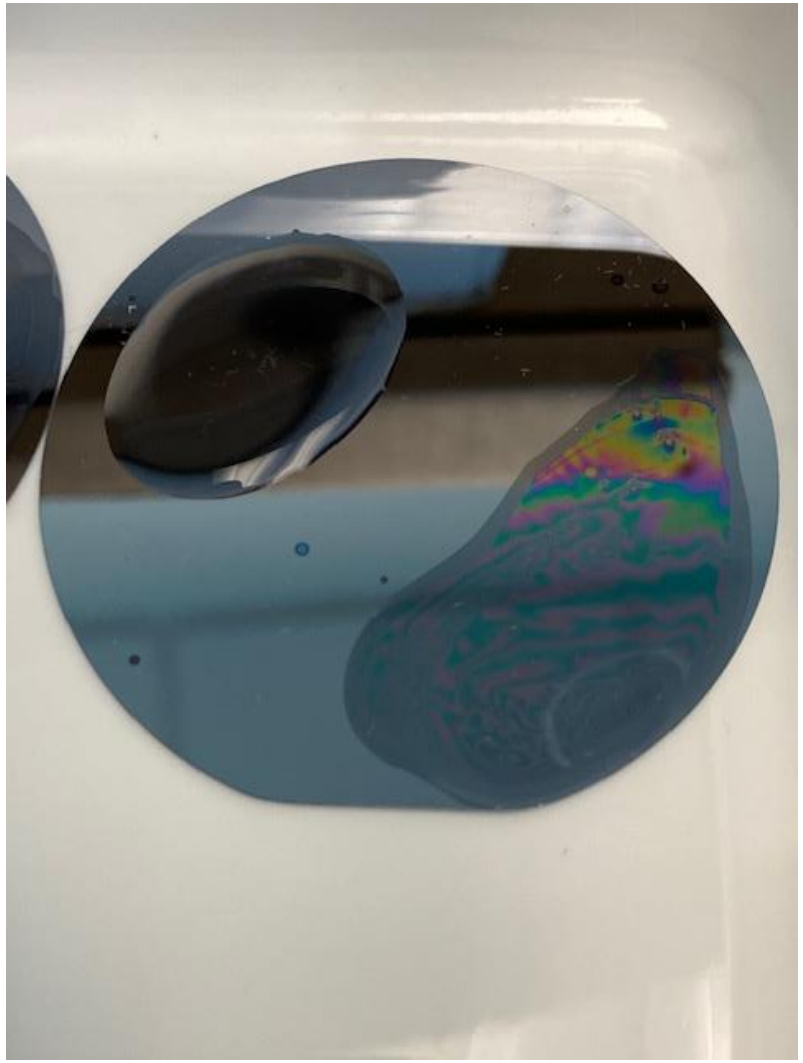
3) Does the ratio of bond breakage and total bond breakage amounts change with a change in particle energy i.e. LET and whether the DNA was in solution or not?

Outline of Experimental Procedures

- Dry DNA samples and hydrated samples (dissolved in distilled water) deposited on 0.6 cm diameter Si wafers were placed at the entrance of a stacked polyethylene phantom (simulating human tissue with a density of 0.96 g/cm3) re: a depth of 0 cm from the base of the PE block as well as in the in the dose build up region at a10 cm depth, as well as at the proton Spread Out Bragg peak (**SOBP**) maximum at 15.4 cm depth and at the SOBP distal 80% depth 15.6 cm from the entrance of the 150 MeV beam.
- Then the samples were irradiated with that 150 MeV clinical proton beam that delivered 1 x 1011 protons/cm2 . Changes to the percentage of molecular bonds in the DNA phosphate backbone and the base pairs were determined using XPS at ARL in Aberdeen, MD.

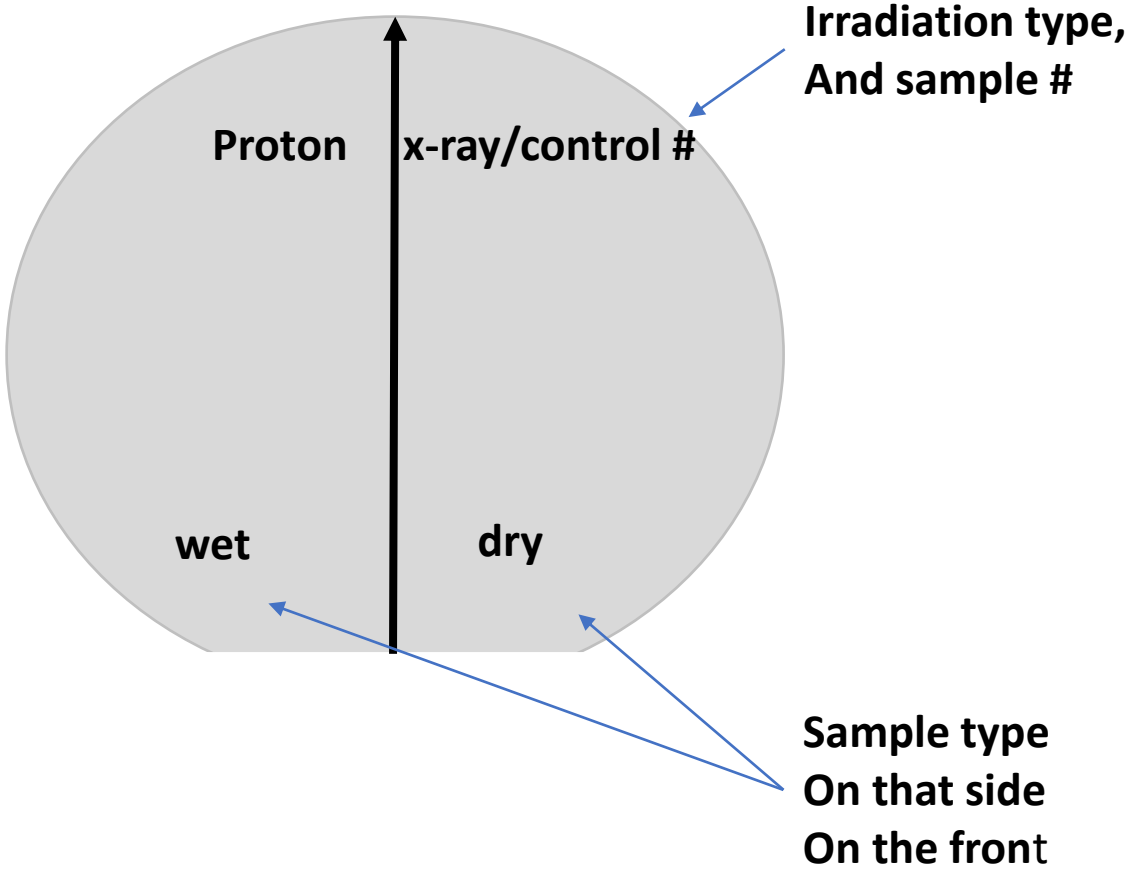
Sample labeling prior to irradiaitons.

Front

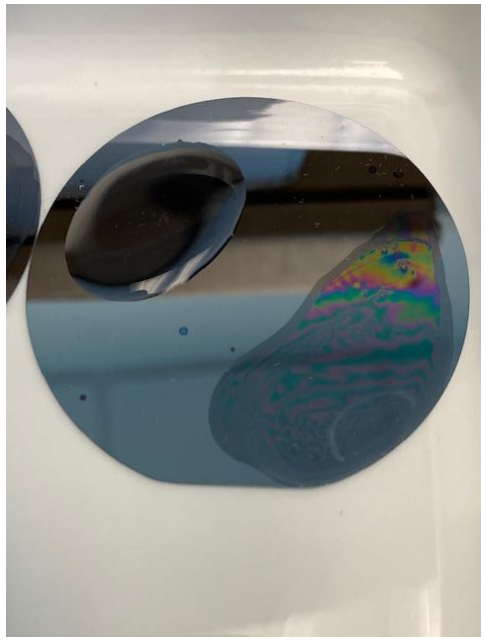


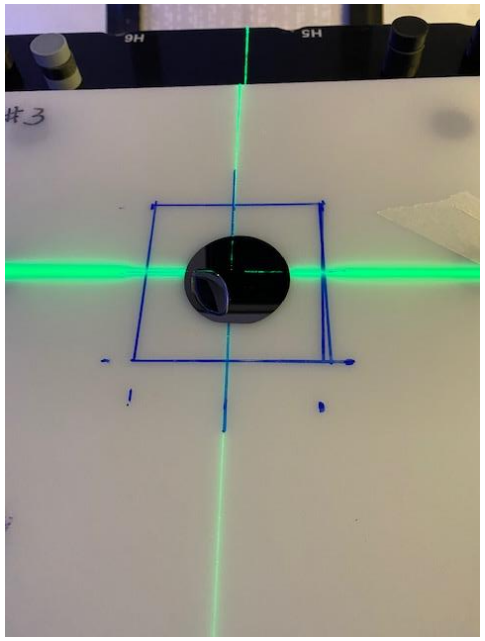
Labeling on the back side of the Si wafer indicated whether the DNA sample on that specific side was wet or dry during the irradiation.

Back of the Si Wafer



Proton irradiations setup at the PTC 11/21/2021



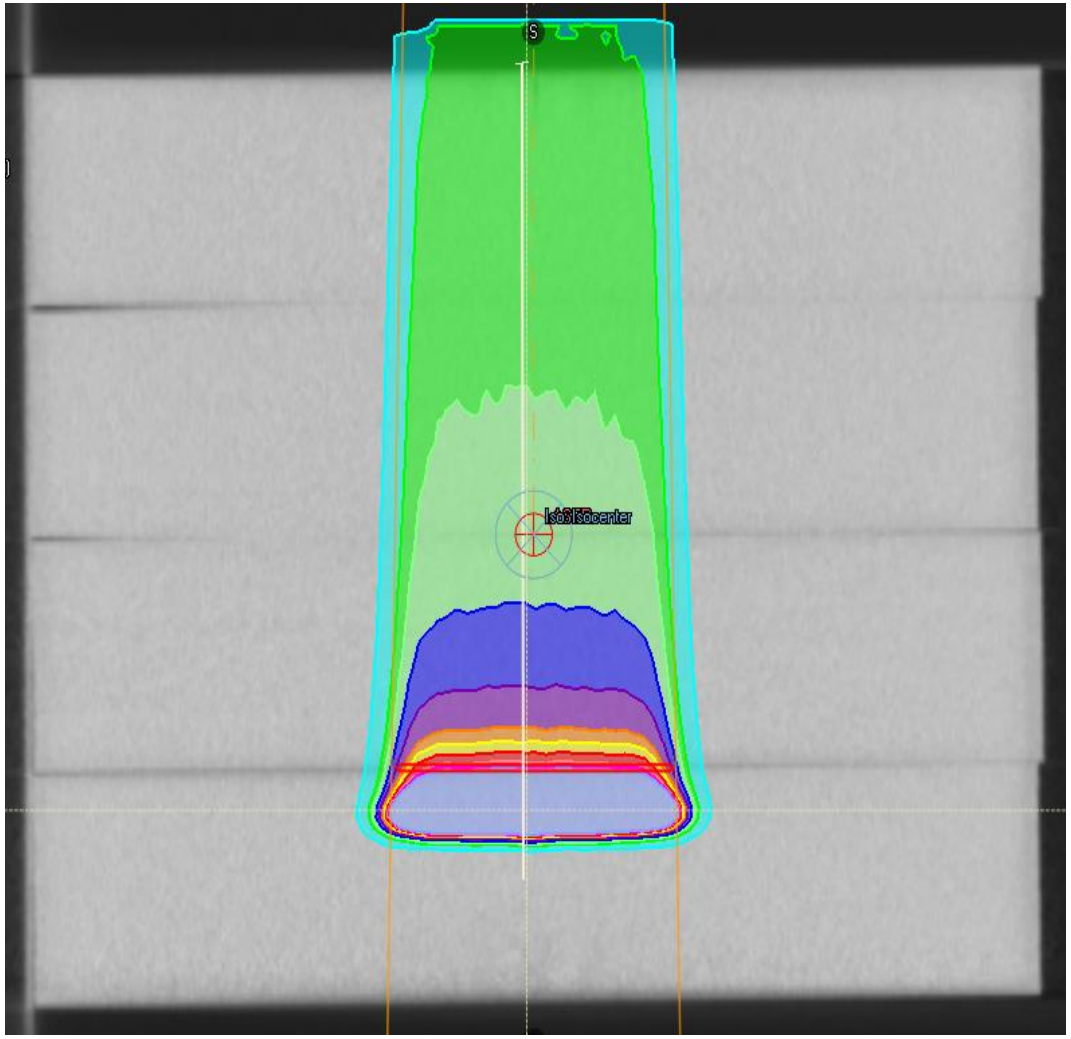


Wet DNA (5 mg DNA/5 ml water) and Dry DNA samples prepared on a 0.1 cm thick Si wafer (Water Eq. Thickness = 0.06 cm). The samples was placed in the center of the 7 cm x 7 cm irradiation field

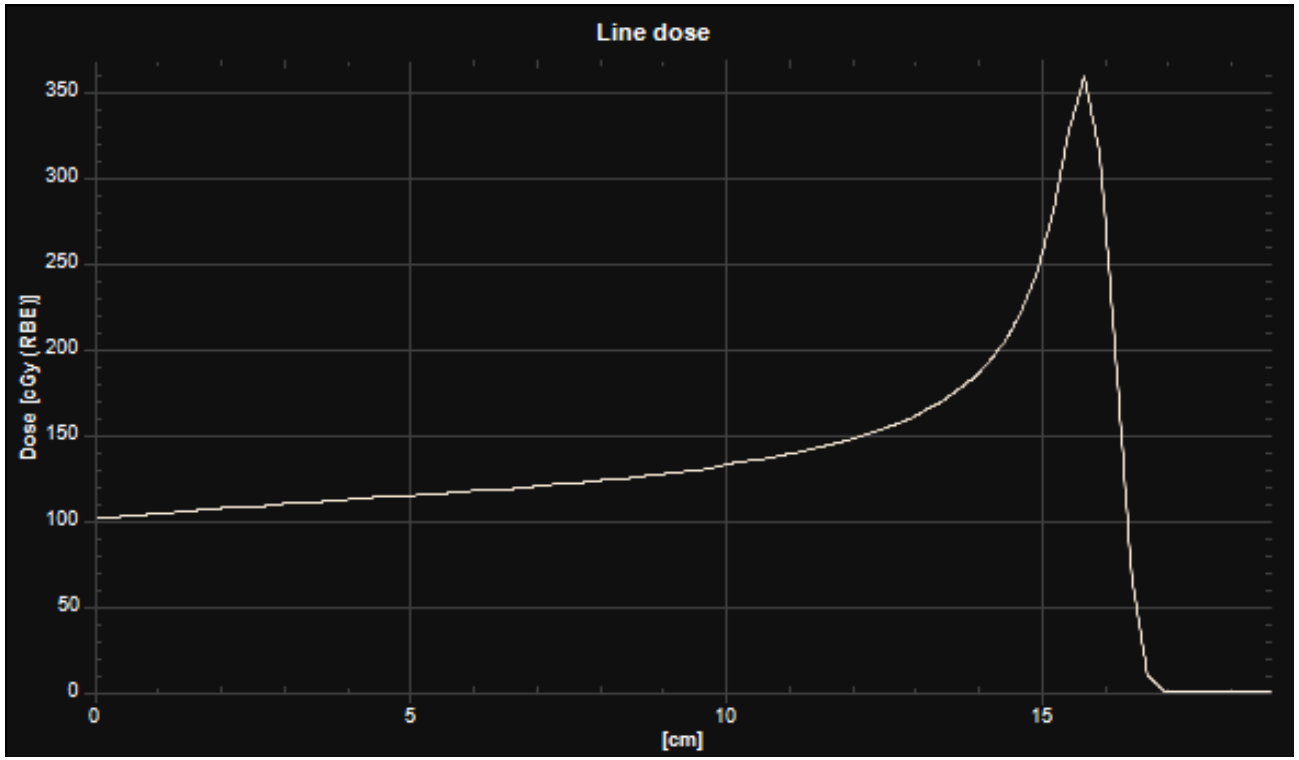
Samples were placed at depths within the tissue equivalent plastic phantom of:

Entrance: (top: 0 cm depth)
10 cm depth: (10.06 cm Depth)
Dose max depth: (15.42 cm depth)
Distal 50% depth: (15.78 cm depth)

A single, mono-energetic proton beam plan was developed to delver a uniform dose and a known number of protons/cm2.

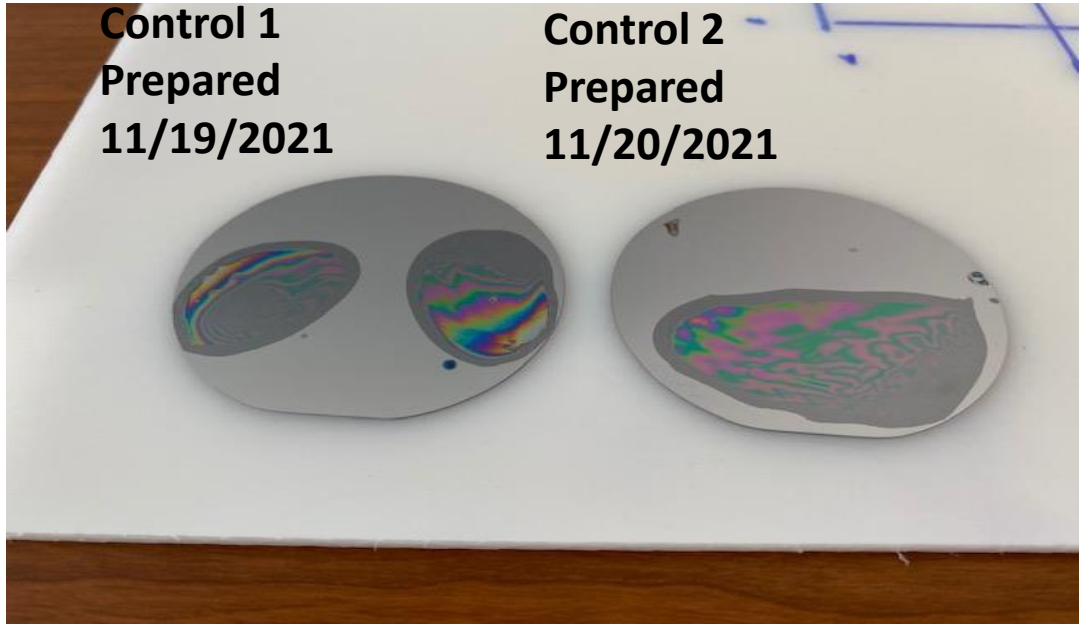


Raystation 9a ION treatment planning system wth a field size: 7 cm x 7 cm
Protons Flux: $1x10^{11}$ protons/cm^2



X-ray irradiation samples 11/21/2021

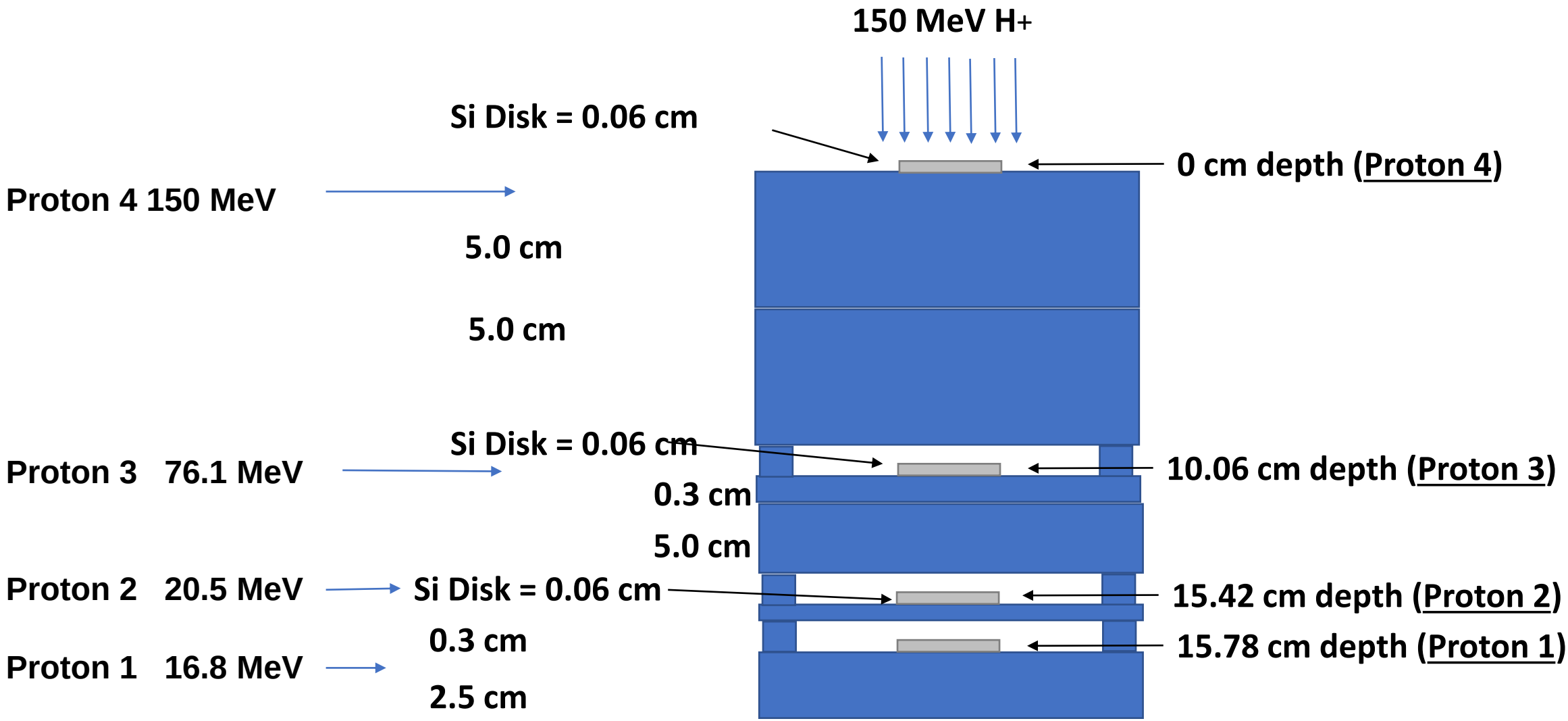
X-ray 2c1000 MU
~10^{12} x-rays/cm2



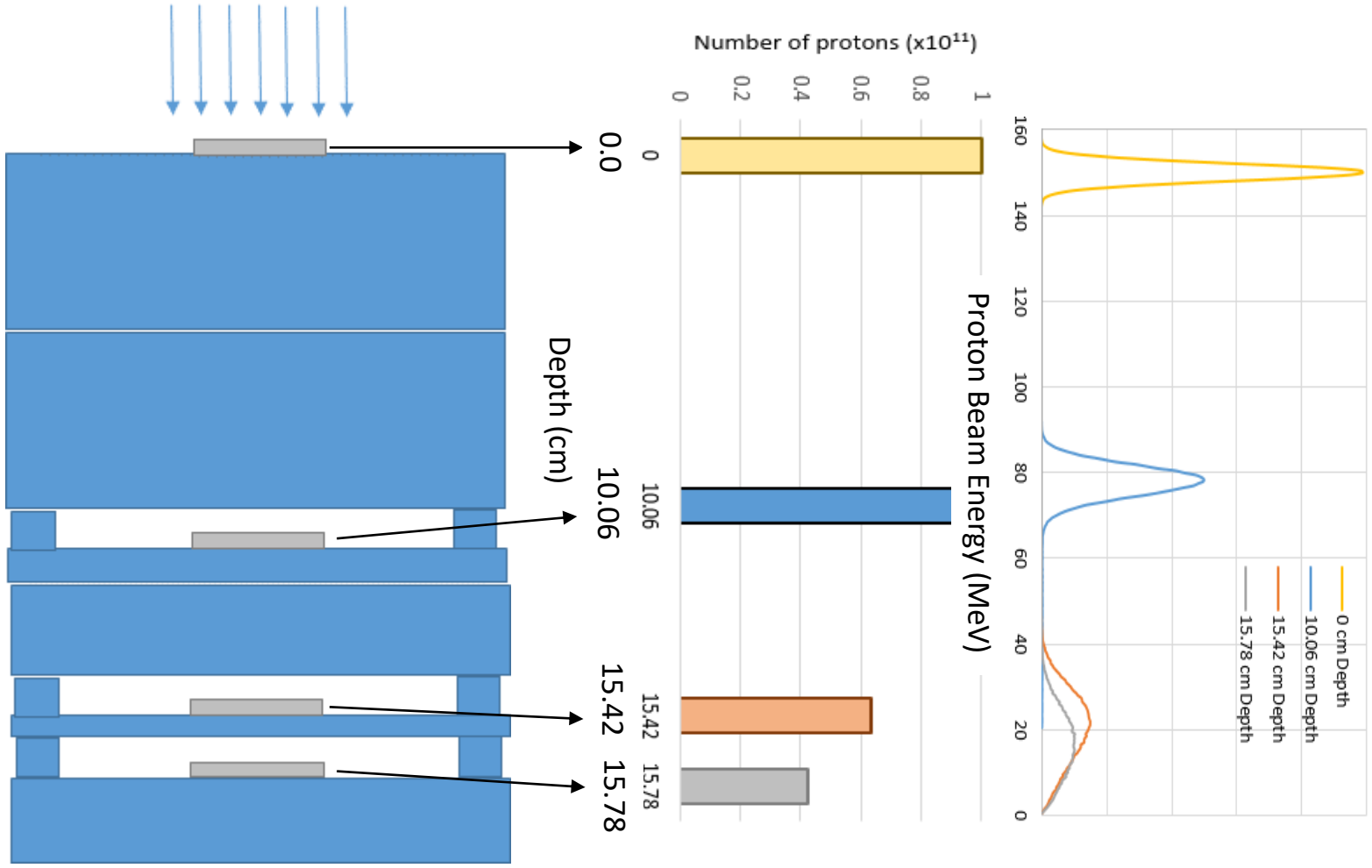
X-ray 1 100 MU
~10^{11} x-rays/cm2

Proton beam delivery parameters and sample setup:

150 MeV incident proton beam, 7 cm x 7 cm field with 1 x 10^{11} protons/cm2

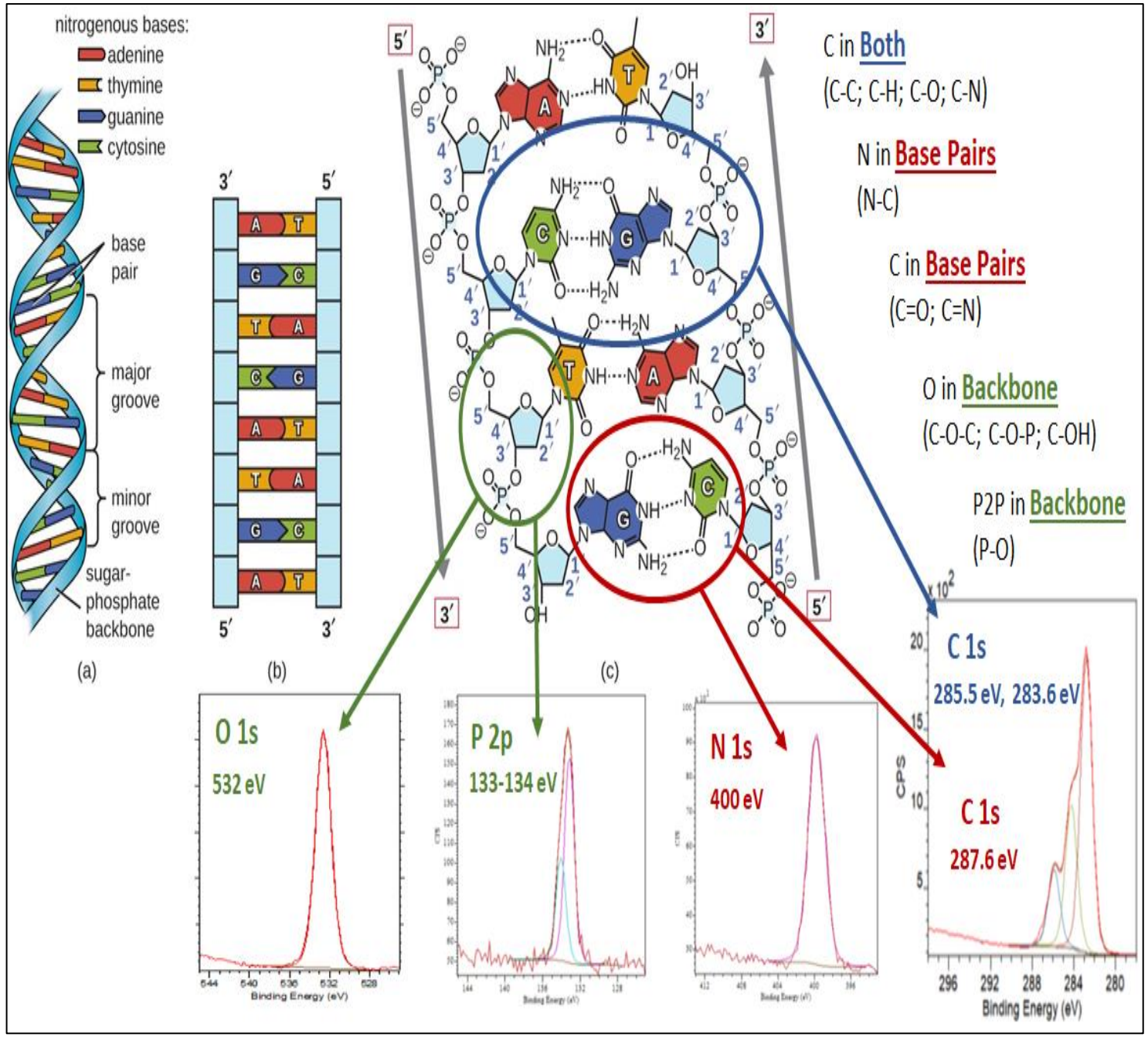


Experimental Detail of the Proton Beam(s) depth for each sample in the phantom



Protons[1]: (x10[11])	Mean Energy[1]: (MeV)	LET[2]: keV/μm	LET_d[3]: keV/μm
1.0	150	0.54	0.39
0.918	76.1	0.89 1.1	
0.637	20.5	2.63	5.76
0.436	16.8	3.25 7.89	

[1]# of protons and Mean Energy determined from Monte Carlo,
[2]LET mean energy taken from NIST [PSTAR] website.
[3]LETd from Raystation 9a ION TPS

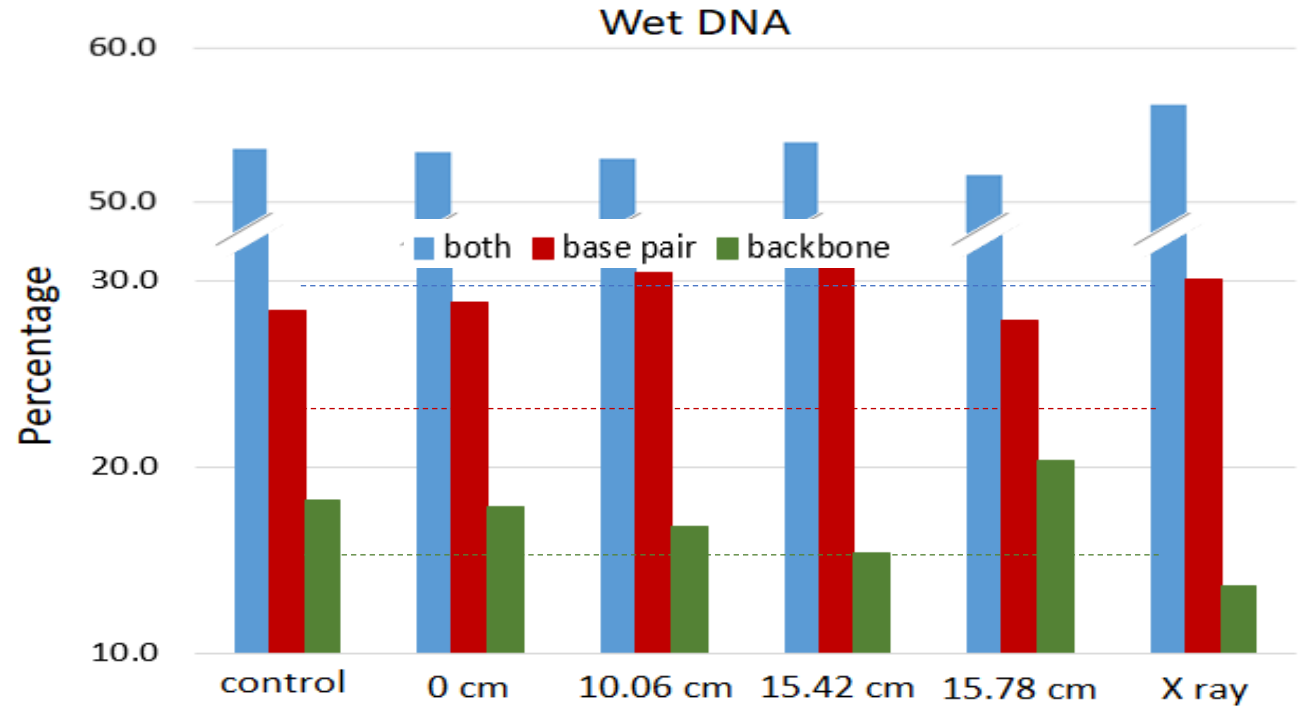


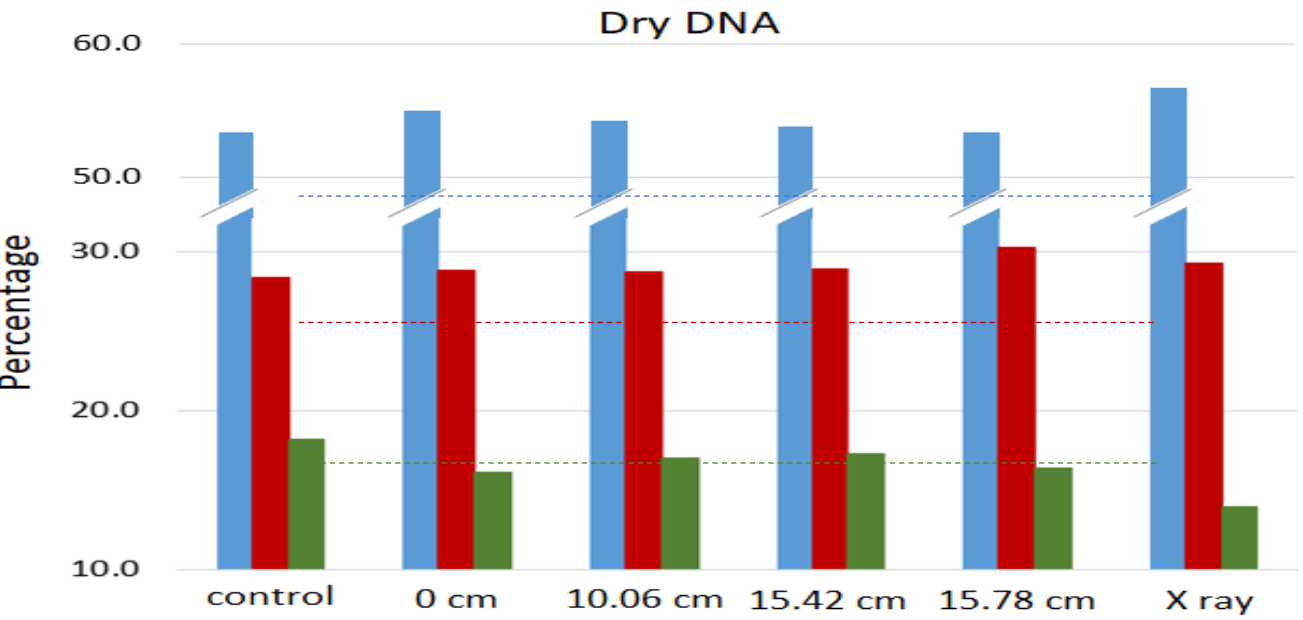
Following irradiations:

X-ray photoelectron spectroscopy (XPS) done at ARL/APG determining the relative number of molecular bonds breaks occurring in DNA during proton beam and x-ray beam irradiation.

Changes in the area under the XPS peaks for energies related to the binding energies for known molecular bond types in DNA. This included XPS signal from:

- Carbon in C-H, C-C, C-N, C-O bonds found in **both** the Backbone and base pairs of DNA.
- Nitrogen and Carbon in N-C, C=O, C=N found only in the DNA **base pairs**, and
- Oxygen and Phosphorus in C-O-C, C-O-P, C-OH, P-O bonds found only in the DNA **backbone**,





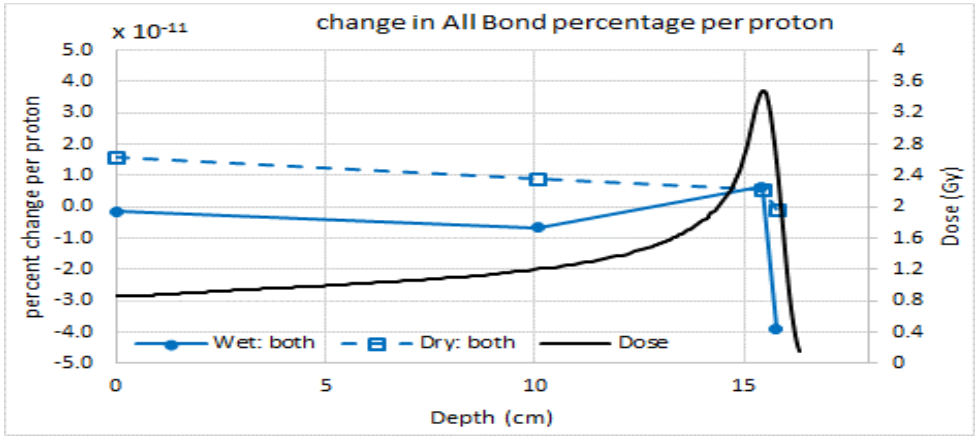
The area under the XPS curve for all molecular bond types (Base pairs, Backbone, Sum = Both) is added together to get the total "molecular bond signal".
Then the percentage of the total for each bond type is determined as: bond type percentage = XPS (bond type)/XPS (total)

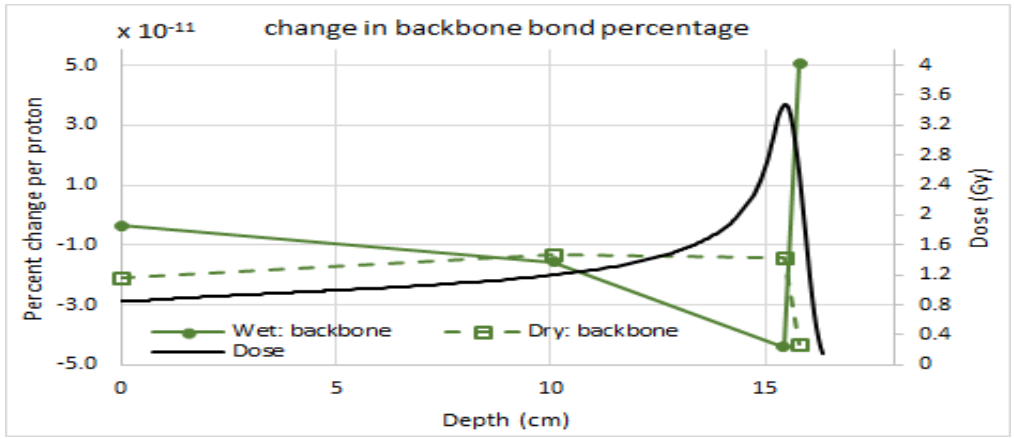
We plotted the sum of the two individual components from Both, Base pairs, and Backbone signals to get the sum percentage for each. We then compare how the sum percentage changes <u>with respect to the control sample</u> for each irradiated sample. We interpret a reduction in the percentage of the Base pair or Backbone as an indicator of the amount of the damage suffered by that location in the DNA. That is, reduction in the percentage of a given bond type (wrt control) indicates loss of those bonds, indicating "radiation damage" to the part of DNA where bonds are located.

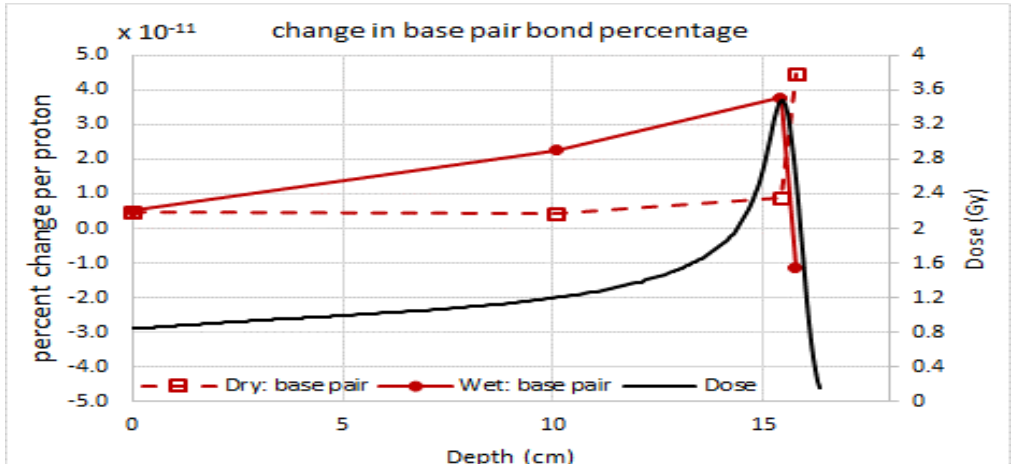
both = C in C-C/C-H % + C in C-O/C-N %

base pair = C in C=O/C=N % + N in N-C %

backbone = O in C-O-C/C-O-P/C-OH % + P in P-O %







DNA damage vs. LET

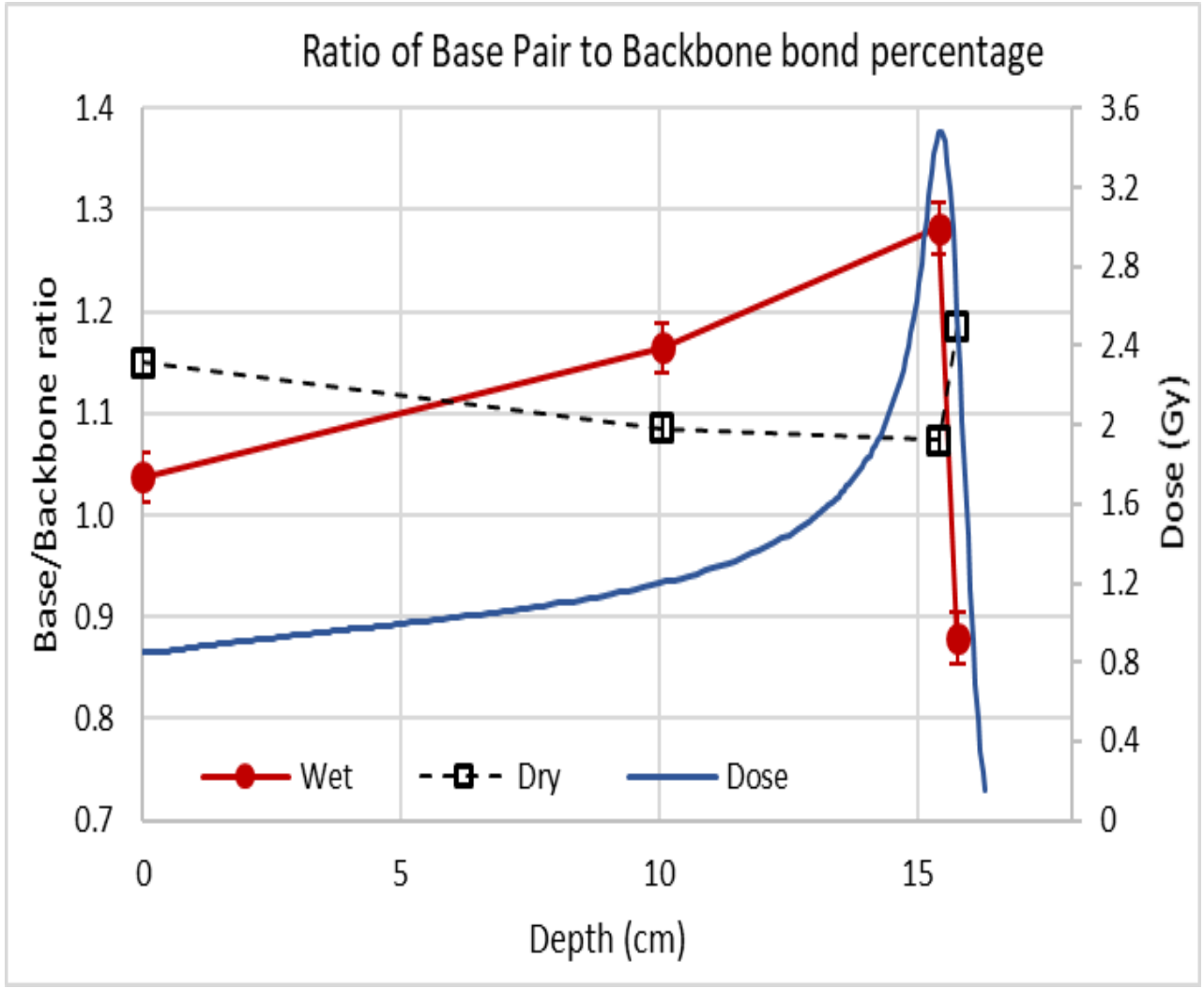
Plotting the change in the bond type percentage (wrt to control) as a function of depth in the phantom along side the proton depth dose profile (*the Spread-Out Bragg peak*) for both the Wet and Dry DNA samples. The bond type changes are normalized per proton since the number of protons incident on the sample decreases with depth in the phantom (***see slide 5).***

All: percentage is relatively stable up to the depth of the ***SOBP*** with only a slight decrease with increasing depth. However, a sharp decrease is seen in the Wet sample beyond the ***SOBP***. This indicates a sharp increase in the amount of damage per proton in the BP and beyond.

Backbone*:* percentage is also relatively unchanged for the Dry sample until beyond the BP where the percentage sharply decreases. For the Wet sample, the percentage decreases as a function of depth until beyond the BP where it increases sharply.

Base pair: percentage increases with depth for the Wet sample and is unchanged for the Dry sample as a function of depth. After the ***SOBP,*** there is a sharp decrease in the WET sample and sharp increase in the Dry sample percentage.

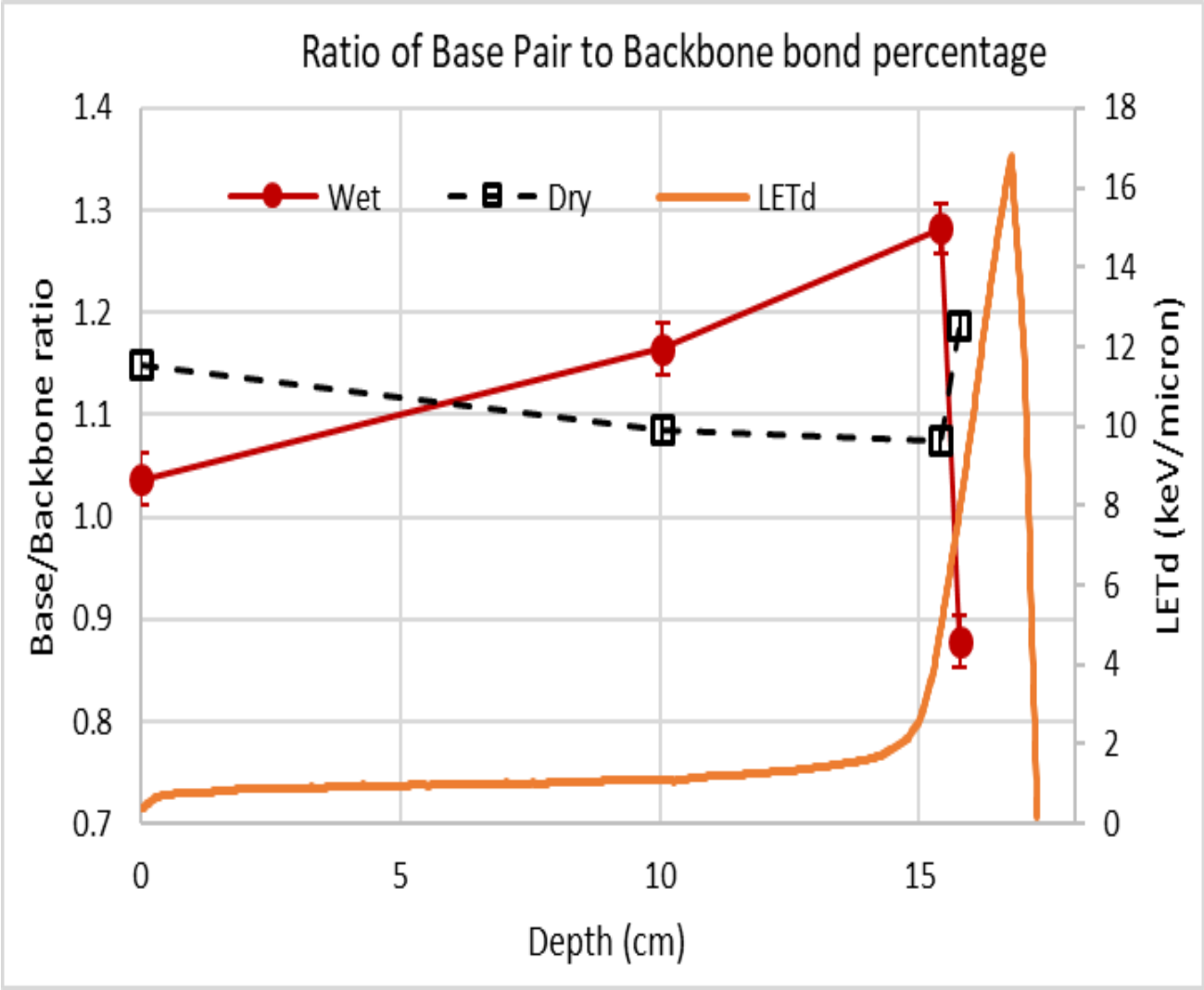
"Preferred" DNA damage type vs depth



Plot of the ratio of base pair to backbone percentage for each sample at each depth plotted against the proton depth dose profile. When plotting this way we see the bond type ratio increases for the Wet sample. This is due (from slide 12) to the reduction in backbone bond percentage further illustrating a preference for backbone bond breaking in the region of proximal to the ***SOBP***

The Wet DNA, beyond the ***SOBP***, the base pair/backbone ratio is less than one, indicating greater base pair damage. Again, indicating the presence of water changes the type of DNA damage that is occurring at the depth of the BP and beyond.

The Dry sample, the backbone damage seems to increase (relative to base pair damage) beyond the ***SOBP*** as the ratio sharply increases.



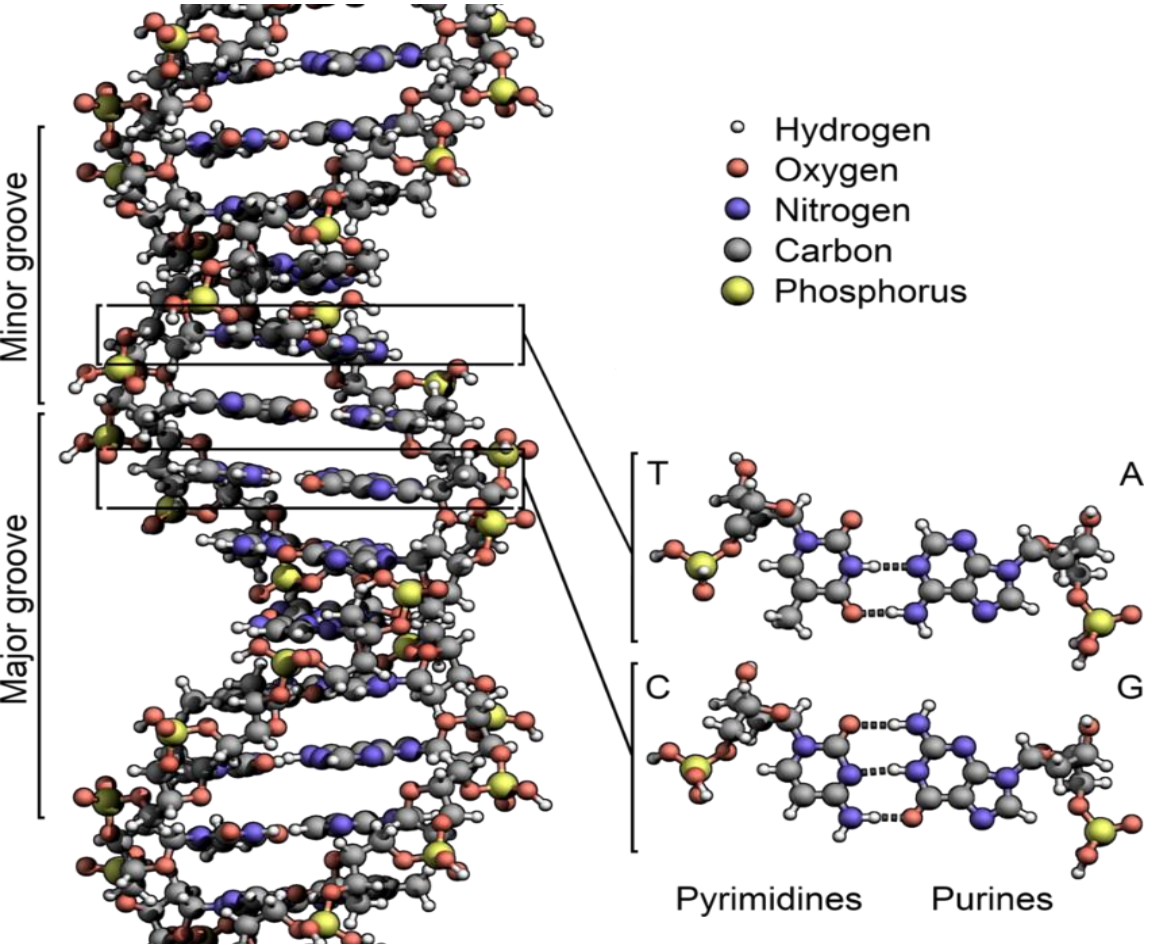
"Preferred" DNA damage type vs depth

Base pair/backbone ratio plotted against the dose averaged LET (LET_d[1]) as a function of depth. In the ***SOBP*** region (15 – 16 cm depth) where the bond damage dynamics change, the LET_d is rising rapidly and could be the cause of the switch from primarily backbone bond loss to base pair bond loss.

In Wet DNA, there is a sharp switch to Base Pair damage in the SOBP region.

[1]LETd from Raystation TPS.

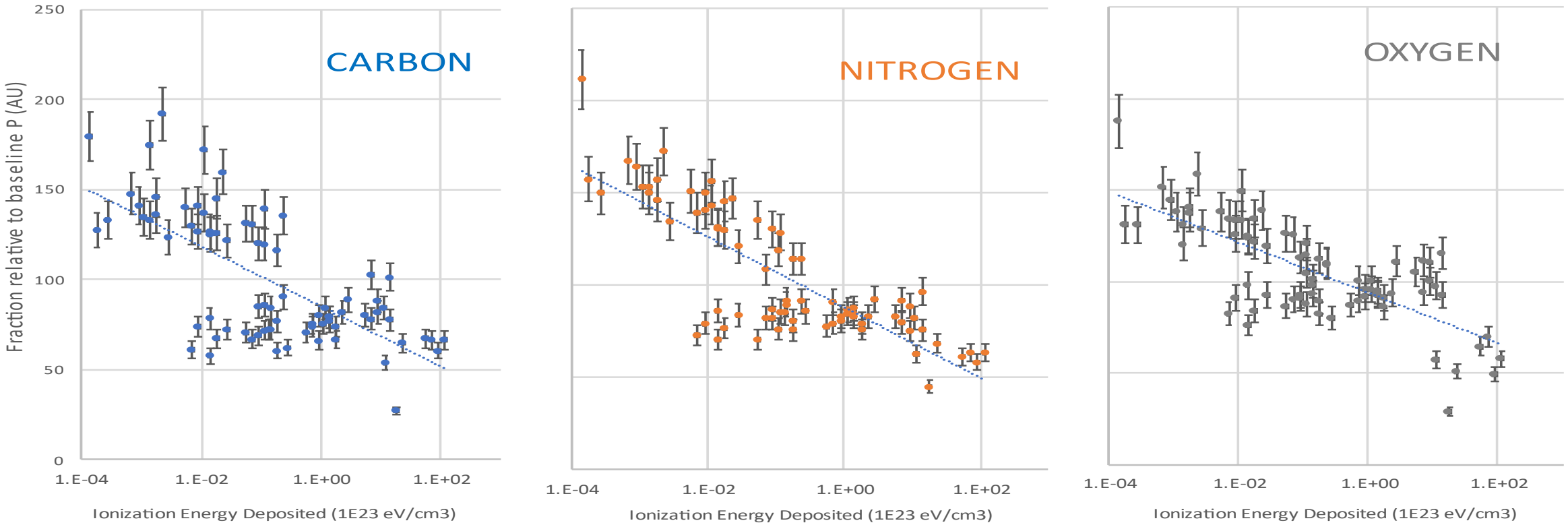
• **Degradation of bovine DNA:** Calf thymus DNA was dissolved in water, spread across a clean silicon wafer, and allowed to dry. H and He ion currents were of <100 nA spread over a 2 mm diameter areas, and fluences varied from 1E13 – 5E15 ions/cm2. XPS was used to evaluate compositional change at the surface after irradiation, normalizing to the amount of phosphorus present, since fragments containing C, N, and O leave the system during vacuum irradiation.



In agreement with previous published studies* using Al x rays, DNA degradation broadly correlated with the total amount of energy deposited in the dried film, regardless of ion species or energy, with evidence that DNA base pairs are preferentially degraded in this circumstance

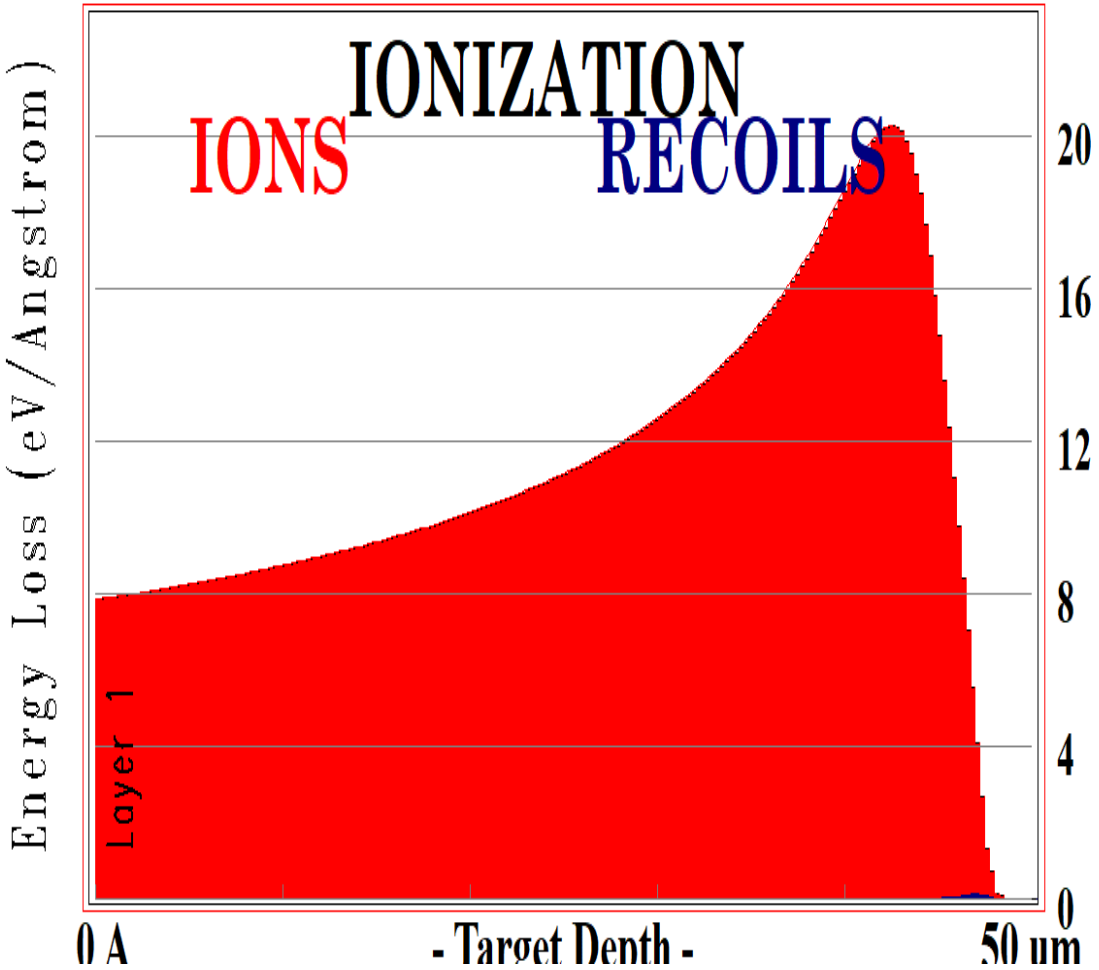
***Hahn 2021, and 2023, Ptsangka 2009**

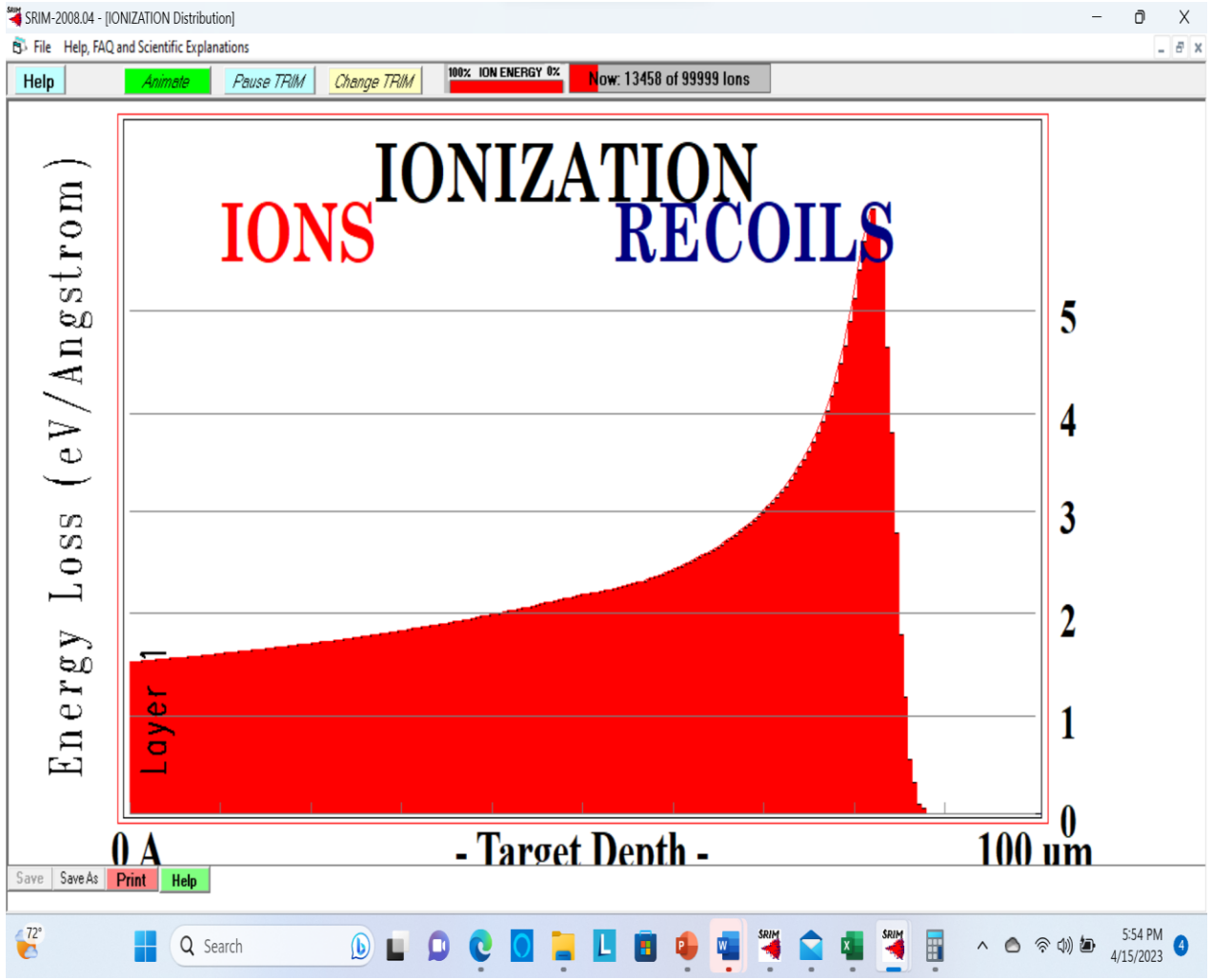
ARL Accelerator irradiations show scaling of N,O,C loss relative to P with Increasing ionization energy deposited, as there indicates slightly more damage to N groups (base pairs) than backbone molecules for only "*dry*" DNA at H and He energies 1-4.5 MeV, LET values range from 11 to 270 keV/μm. In reality the *'dry*" DNA would be very hydrated with exposure to air humidity



Lowest doses would be ~ 900 Gy i.e. 3 MeV protons, highest would be 1 MeV alpha particles ~ 1 Ggy delivered with beam current 50-100 na. Recent work at the ARL/APG tandem accelerator has beam currents down to 0.01-0.1 na so the delivered doses can now be taken down to the 1-100 Gy range routinely.

EOR and SOBP for 5.5 MeV α's typical of Alpha Therapy drugs and 2.0 MeV protons related to Fission Peak neutron forward scattering

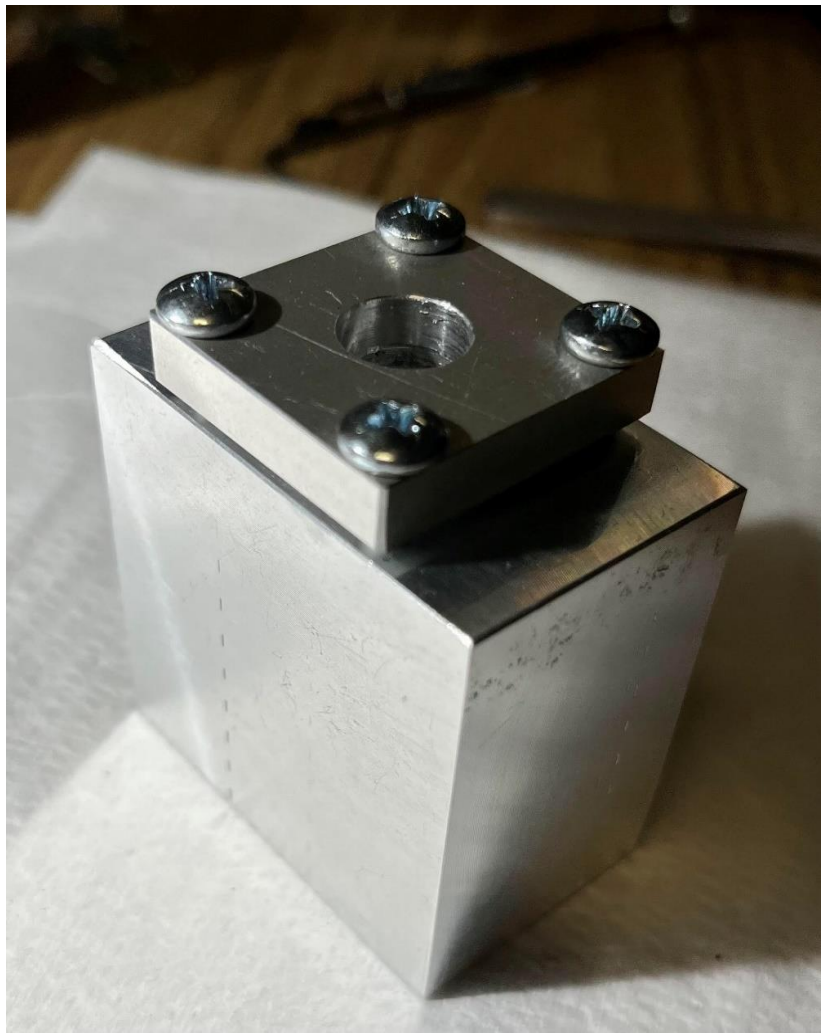




5.5 MeV α's in H2O max energy from 225Ac & 223Ra, for TAT. SOBP ranges from ~ 0.400 – 4.50 MeV, 4-30 μm EOR with LET's 80-278 keV/μm

2 MeV Protons on H2O corresponding to recoils from the peak in the fission spectrum SOBP ranges from ~90-900 keV and 1.5 – 21 μm EOR with LET's 30 to 90 keV

Future Measurements in Aqueous Medium



Liquid sample irradiations with high energy protons at the PTC, fast neutrons and ARL APG accelerator and also characteristic ϒ-rays from 0.500 – 7.5 MeV as well as Auger electrons and x rays.

Made of Al can be used in our outside of a vacuum chamber has a total volume of 3.8 cm3 but it can be adjusted to accommodate different particle path lengths in terms of what portion of a particle's 1D path length can be investigated in terms of its effect on a material dissolved in water in this sample holder. For uncharged particles like fast neutrons or gamma rays the entire volume of the holder can be used for irradiations at different energies and intensities. A similar holder made out of PMMA to be more tissue equivalent can be fabricated for future studies.

It can be used to study End-Of-Range effects with high-energy protons from the Medical Cyclotron at the Univ. of MD Medical Center's Proton Therapy Facility in Baltimore MD by adjusting the amount of liquid placed inside along with dissolved DNA samples. For fast neutron. X rays, Auger electrons and ϒ's a PMMA based holder would be preferable in terms of a more realistic tissue environment.

Summary and Conclusions

- ***In the entrance 150 MeV(0.55 kev/μm) and build up 76.1 MeV (0.91 kev/μm) regions the dominant form of DNA damage is through phosphate backbone bond breaks for both dry and hydrated DNA. However, at the depths of the SOBP maximum (~2.6 keV/μm linear energy transfer (LET)) and distal 80% falloff (LET ~3.1 keV/μm), backbone bond damage increased sharply for dry DNA, however for hydrated DNA base pair bond damage increases and becomes dominant.***
- ***Overall the percentage of bond break types found in both DNA backbone and in base pairs is relatively stable up to the depth of the Spread Out Bragg peak (SOBP) with only a slight decrease with depth in the phantom. However, a sharp decrease is seen in the WET sample at these same bombarding conditions.***

- ***,This relatively sharp increase in the base pair damage in the hydrated DNA in the high LET BP region couldprovide information as to the importance of reactive oxygen species created in water in the proves of DNA damage by proton irradiation. Additionally, the increasing base pair damage might provide information as to why the relative biological effectiveness of protons beams increases as LET increases near the end of range.***

Summary of Results

For Irradiated Wet DNA

- Base pair damage increases (**i.e. base pair % decreases**) for LET > 2.6 keV/um in the vicinity of the **SOBP**. Our overall results are in good agreement with Hahn's who used 1.5 keV x rays (Al K x rays)

As LET increases, the production of OH-decreases (Hahn 2021 and Ptasinlga 2008). Since OH- preferentially attacks the backbone* we see a decrease in backbone damage, and thus an increase in the backbone bond % and decrease in base bond % as evidenced by the loss in N while P content remains unchanged.*

- The difference in Backbone vs Base Pair damage in the ***SOBP*** region seems to be much larger than the difference for x-ray irradiated DNA. In fact, after the **SOBP** (the higher LET region), there is a sharp decrease in base pair bond percentage for the WET sample and a sharp increase in the DRY sample percentage.

- Is this a major factor for the increase in RBE of protons in the region of the ***SOBP?***

*Hahn, M.B., Dietrich, P.M. & Radnik, J. *Commun Chem* **4,** 50 (2021). https://doi.org/10.1038/s42004-021-00487-1

Ptasinskga, S, et al, Journal of Chemical Physics, **129(**6) (2009), pp. 129–134, http://dx.doi.org/doi:10.1063/1.2961027

Some Additional Notes of Interest

- The SOBP is roughly 50 MeV wide for 150 MeV protons through PE as opposed to lower energy irradiations where it would be 0.09 – 4.5 MeV. Much finer slices of LET would be possible.
- Doses to "*dry*" DNA* at ARL are in the 900 kGy to 100 MGy region using ~ 100 na of beam current. Presently experiments can be done there with 1 x 10-3 to 10-4 less beam on target bringing delivered doses down to the 1-10 Gy region.
- To do irradiations at these lower beam energies at ARL in H2O is challenging because samples on the order of 0.003 – 0.015 ml would be needed to have an EOR total irradiation of DNA in solution.

The "dry" samples used at both PTC in Baltimore and ARL at Aberdeen are not in reality dry in terms of moisture Content. It is just that they are not irradiated in aqueous solution so the amount of water molecules present is much lower than for the dissolved DNA experiments performed at PTC. Aqueous samples can not be irradiated at ARL accelerator because all the irradiations are done in high vacuum system to allow the ion beam to hit the DNA